# Role of Interleukin-8 in Periodontal Disease

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## Abstract

**AIM & OBJECTIVE**: a) To estimate the concentration of IL-8 in health, in plaque associated gingivitis and in chronic periodontitis.b) To correlate the concentration of IL-8 with other clinical parameters of gingival and periodontal status.c)To determine if IL-8 can act as a marker/indicator in chronic periodontal disease destruction. **STUDY DESIGN**: A total of 75 systemically healthy patients, aged 25-50 years were included in the study. Based on the clinical evaluation and radiographic examination, the patients were divided into 3 groups - healthy, plaque associated gingivitis and chronic periodontitis. Gingival Crevicular fluid from these patients was collected using micro-capillary pipettes and the concentrations of IL-8 in the samples were determined using ELISA. **RESULTS**: IL-8 was detected in all three groups. In periodontally diseased subjects, the concentration of IL-8 was significantly elevated when compared to healthy subjects. **CONCLUSION**: The results suggested that gingival crevicular IL-8 levels increases with the increase in periodontal destruction. Based on the results of this study and previous clinical trials this article discusses the relevance of IL-8 in chronic periodontitis and its role as a biological marker.

**Key words:** IL-8, Plaque induced Gingivitis, Chronic Periodontitis, ELISA, Gingival Crevicular fluid

## Introduction

Periodontal diseases are a group of inflammatory conditions of microbial origin that results in the destruction of epithelial and connective tissue attachment and the supporting structures around the teeth. The initiation and progression of periodontitis are dependent on the presence of various virulent microorganisms in the supra gingival and subgingival plaque – that acts as a microbial biofilm [2].Out of these the most significant are the gram negative anaerobic microorganisms present in the sub gingival region that typically include Porphyromonas gingivalis, Treponema denticola, Aggregatibacter actinomycetemcomitans and Tanerrella forsythia. These microorganisms possess an array of virulence factors that enhance the infectivity and provide the ability for the organism to multiply and persist in periodontium [4]

The microbial challenge results in the activation of host response. The host responds with an immediate inflammatory and immune response through the activation of complement cascade, resident leucocytes and mast cells. The neutrophils function primarily as antimicrobial cells, where as the chronic inflammatory cells orchestrate adaptive responses. Leukocyte migration is essential for immune surveillance of tissues by focusing immune cells to the sites of antigenic challenge[3].

The complex interaction among inflammatory cells and other cellular elements in connective tissue is mediated by a series of low molecular weight proteins called cytokines.Interleukin-8(IL-8) is one such cytokine that acts as a powerful chemoattractant for neutrophils and has been identified in crevicular fluid[5,6,8].

IL-8 is a potent activator of human PMNs as this cytokine induces shape changes of neutrophils resulting in pavementing and diapedesis;promotes chemotaxis,and causes a rise in intracellular free calcium, potentiating the respiratory burst and exocytosis of primary and secondary granules from these cells[8].IL-8 also induces rolling arrest and adhesion of PMNs to endothelial cells, facilitating transendothelial migration[7].

The levels of IL-8 in gingival crevicular fluid - as a possible biomarker for periodontal disease was assessed in various studies[3,9].They concluded that the amount of IL-8 is increased in sites of increased periodontal break down when compared to healthy sites. On the other side several studies on the level of IL-8 in chronic periodontitis [8,10]indicated a higher level of IL-8 in healthy sites when compared to sites of increased periodontal break down. This disparity on the level of IL-8 in periodontal health and disease has resulted in confusion, as to whether an increase or decrease in IL-8 would serve as a biomarker for periodontal breakdown.

This study aims to assess the levels of IL-8 in health as well as in periodontal disease with the goal of lending greater clarity on the possibility of IL-8 being used as a biomarker, for active periodontal break down.

## MATERIALS AND METHODS:

The 75 patients who have taken part in the present study had been chosen from among the patients who are attending the outpatient Department of Periodontology and Oral Implantology clinic of SRM dental college, Chennai for the management of their dental and oral conditions. Approval was taken from the ethical committee of SRM Dental College Chennai,for carrying out the study.Subjects were classified into three groups based on clinical parameters such as plaque index, gingival index, bleeding on probing, probing pocket depth (PD), attachment loss (AL) and bone loss.

**Group I (control group) n=25:** Periodontally healthy subjects characterized by clinically healthy gingiva, absence of bleeding on probing, probing depth ≤3.0mm with no clinical attachment loss.

**Group II (Plaque associated gingivitis group) n=25:** Characterized by presence of bleeding on probing, probing depth ≤3mm with no clinical attachment loss.

**Group III (Chronic periodontitis group) n=25:** characterized by presence of bleeding on probing, pocket depth ≥ 6mm, clinical attachment loss ≥5mm.

## SELECTION CRITERIA

**Inclusion Criteria** – Systemically healthy patients in the group between 25-50 years who had not received any antibiotic therapy and periodontal therapy in the last 6 months, having a minimum number of 20 teeth, excluding 3rd molars with not more than 2 teeth missing in each quadrant were included in the study. All the subjects were explained about the study and an informed consent was obtained from the patients.

**Exclusion Criteria** – Pregnant and lactating women, smokers, patients with chronic inflammatory diseases and patients with acute infections were excluded from the study

## METHOD OF SAMPLE COLLECTION:

**PROCEDURE FOR GINGIVAL CREVICULAR FLUID COLLECTION[11]** Subjects selected for the study were made to sit comfortably in an upright position on the dental chair with proper illumination.Crevicular fluid was obtained by placing calibrated volumetric micro-capillary pipettes(Obtained from Sigma Aldrich) at the gingival margin. A standardized volume of (*100 microlitre*) crevicular fluid was collected by placing the tip of the pipettes extracrevicularly. Samples of gingival crevicular fluid contaminated by blood or saliva were discarded.

## CENTRIFUGATION AND STORAGE

The samples were immediately transported to the laboratory at the Blood Bank- Sri Ramachandra Medical College where the samples were centrifuged and the supernatant stored at -70 degree celcius.The samples were then assayed for IL-8 concentration by using the Quantikine human IL-8 ELISA kit (Obtained from R&D system Minneapolis, MN USA).Samples were analysed in the Blood Bank of Sri Ramachandra Medical College and Research Institute, Porur Chennai.

## STATISTICAL ANALYSIS:

Mean values were compared among different study groups by using Kruskal-Wallis one way ANOVA followed by Mann-Whitney U test. Bonferroni correction procedure was employed to adjust the p-values for multiple comparisons. Spearman Rank Correlation analysis was done to assess the relationship between various study parameters.

In the present study, $p<0.05$ was considered as the level of Significance.

## RESULTS:

A total number of 75 subjects comprising of 45 males and 30 females participated in this study throughout. The study was carried out among South Indians (Chennai population). Subjects were classified into three groups based on clinical parameters such as plaque index, gingival index, bleeding on probing, probing pocket depth (PD), attachment loss (AL) and bone loss. A significant difference ($p<0.0001$)was found in all the clinical parameters between group I and II, between group I and III and between group II and III.The mean IL-8 concentration was 1.056±1.845pg/ml in group I, 6.021±2.327pg/ml in group II and 35.199±8.815pg/ml in group III. A significant difference ($p<0.0001$) was found in the IL-8 concentration between group I and II, between group I and III and group II and III.

Statistically significant differences were obtained between Group 1,2,and 3 regarding the level of IL-8 and other clinical parameters.

## CORRELATION BETWEEN DIFFERENT VARIABLES

Spearman's rank correlation test to look for any correlation between the gingival crevicular fluid IL-8 concentration and clinical parameters, showed a significant positive correlation except for pocket depth and neutrophils count.

**Table 1**: Comparison of IL-4 concentrations among the groups using the Kruskal–Wallis test and the Mann–Whitney U-test

| Variable | Group 1 Mean±S.D | Group 2 Mean±S.D | Group 3 Mean±S.D | p-value |
|---|---|---|---|---|
| | n=25 | n=25 | n=25 | <0.0001 |
| **OHI-s** | 0.06±0.13 | 1.38 ±0.39 | 5.71±0.39 | <0.0001 |
| **Plaque index** | 0.04±0.01 | 1.01±0.15 | 1.98±0.07 | <0.0001 |
| **Gingival index** | 0.0±0.0 | 2.0±0.0 | 2.0±0.0 | <0.0001 |
| **Bleeding index** | 0.0±0.0 | 3.0±3.0 | 3.0±3.0 | <0.0001 |
| **Neutrophil %** | 58.8±1.7 | 64.6±0.8 | 68.9±1.3 | <0.0001 |
| **Pocket depth** | 0.9±0.3 | 3.1±0.3 | 7.0±0.5 | <0.0001 |
| **Cal** | 0.0±0.0 | 0.0±0.0 | 4.4±0.6 | <0.0001 |
| **IL-8** | 1.056±1.84 | 6.021±2.327 | 35.199±8.81 | <0.0001 |

*OHI-Oral Hygiene Index, Cal-Clinical Attachment Loss, IL-8-Interleukin- 8*
*Mean±S.D-Mean ± Standard deviation,n-Number of subjects*
*p-Value-value of significance.*

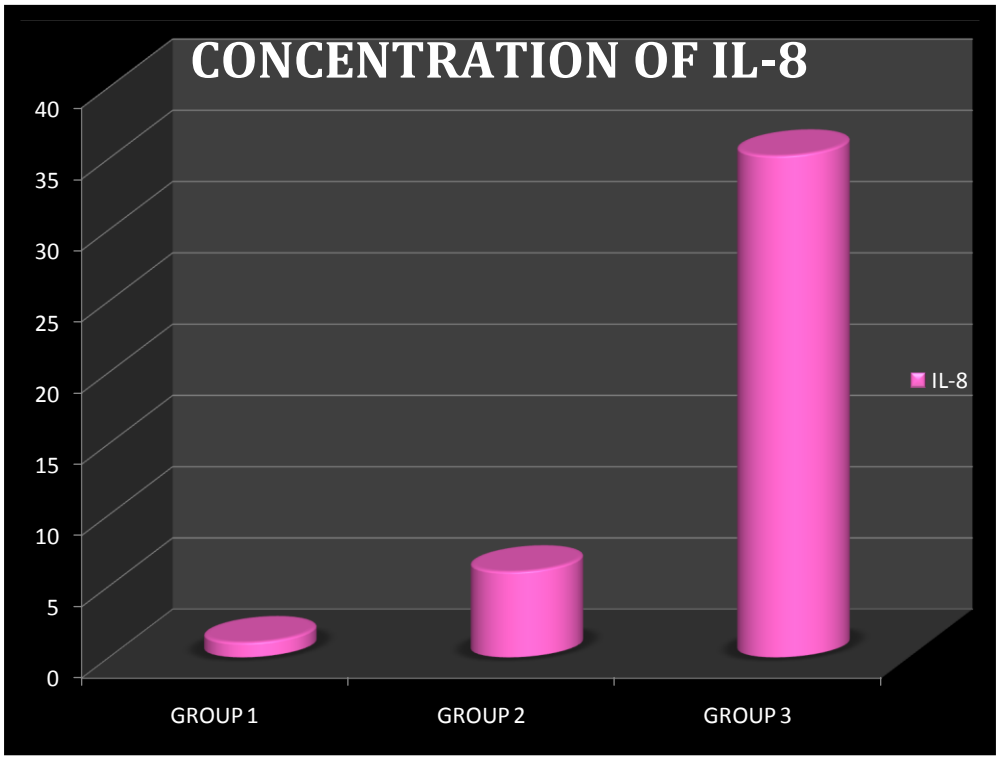


**Figure 1**: Comparison of the total concentration of IL-8(pg/ml) between group 1(health/n=25), group 2(plaque associated gingivitis/n=25) and group 3(chronic periodontitis /n = 20). Statistically significant differences were obtained between Group 1, 2, and 3 regarding the level of IL-8 ($p < 0.05$).

**Table 2:** Spearman's Rank Correlation ('r') Coefficient

| Variables | Group 1 Correlation coefficient ('r') | Group II Correlation coefficient('r') | Group III Correlation coefficient('r') |
|---|---|---|---|
| **OHI** | +0.02* | +0.45* | +0.05* |
| **Plaque index** | +0.04* | +0.14* | +0.07* |
| **Neutrophil (%)** | -0.38* | -0.03* | -0.40* |
| **Probing depth** | -0.33* | -0.34* | +0.09* |
| **Clinical Attachment Loss** | | | +0.30* |

**Indicates if the correlation co-efficient value is between 0 and 0.5, there is a weak correlation; between 0.5 and 1, there is a strong correlation and if the value is 1, there is perfect correlation. The '+' or '–') sign indicates that the correlation is positive or negative respectively.*

## DISCUSSION

Chronic periodontitis is characterized by inflammatory destruction of connective tissue, loss of periodontal attachment, and resorption of alveolar bone.[12]Current understanding of the pathogenesis of periodontitis suggests that it is of a multifactorial nature, being a result of complex interactions between pathogenic subgingival microorganisms and the host response.

The goal of all periodontal diagnostic procedures is to provide useful information to the clinician regarding the disease type, location and severity. Traditional diagnostic procedures provide useful information to the clinician regarding the disease type, location and severity. But traditional diagnostic procedures are inherently limited[13] in that only disease history and not current disease status can be assessed. Increasing knowledge of these complexities provides new opportunities for diagnostic strategies to be investigated.

Various clinical trials have been attempted to identify and quantify markers that indicate risk for future periodontal break down.[14,15,16] Studies have been attempted on indicators of periodontal disease activity. Host derived biochemical enzymes such as Proteolytic enzymes[17],hydrolytic enzymes[18,19], extracellular matrix components [20],bone forming markers[21,22],bone resorption markers[23], micro- biological markers[4,24,25],etc have been studied to indicate periodontal disease activity.

IL-8 is a cytokine /chemo attractant protein/chemokine which is produced by a variety of immune inflammatory cells in response to inflammation[27].IL-8 functions primarily to activate neutrophils, and plays a role in PMN recruitment to the inflammatory site[6,26,29].

The current study attempted to quantify IL-8 to evaluate its role in periodontal diseases. Gingival crevicular fluid was taken from patients categorized into healthy, plaque associated gingivitis and chronic periodontitis groups.IL-8 concentration was assayed using Quantikine human IL-8 ELISA kit. The study was carried out on 75 patients, categorized as healthy (25), plaque associated gingivitis (25), and chronic periodontitis (25). Systemically healthy patients between 25-50 years of age, who had not received any antibiotic and periodontal therapy were included in the study.64% were males and 36%females in control group, 60% were males and 40% females in gingivitis group and 56% were males and 44% females in periodontitis group were included. Clinical parameters like calculus, plaque, probing depth, gingival bleeding, gingival inflammation and clinical attachment loss were assessed. Sites for sample collection for group II and III had Gingival Index score 3,Gingival Bleeding index score,3 and probing depth ranging from 0-3mm in group I;3-4mm in group II;and 6-8mm with mean clinical attachment loss of 4mm in group III.

The mean IL-8 concentration was 1.056±1.845pg/ml in group I,(Healthy),6.021±2.327 pg/ml in group II(Plaque associated Gingivitis),and 35.199±8.815pg/ml in group III.(Chronic Periodontitis).A significant difference ($p<0.0001$)was found in IL-8 concentration between group I & II,group II & III and group I & III. An increased concentration of IL-8 was significantly associated with diseased sites. Clinical parameters like plaque index and probing depth were positively correlated with IL-8 concentration. Our results indicated that elevated concentrations of IL-8 in GCF are associated with sites showing periodontal destruction. These levels exhibited statistically highly significant difference between the three groups.

Data concerning the association of IL-8 in GCF and severity of periodontal inflammation have been equivocal. Two studies suggested an inverse relationship between IL-8 activity and PMN recruitment. Chung et al in 97[8] observed an inverse relationship between the concentration of IL-8 and recruitment of PMNs to the diseased sites. Tonetti et al 93[10] observed a reduced gene expression of IL-8 in gingival tissue biopsy taken from periodontally diseased sites.

On the contrary many other studies along with the present one suggested a positive relationship between GCF IL-8 levels and periodontal disease. According to[11]Mathur et al 96,[9]Cheng et al 97 and [31]Giannopoulou et al 03, the concentration of IL-8 was significantly higher in GCF from patients with diseased sites when compared to healthy sites. More over [28]Dongari et al 96, observed that expression of IL-8 in gingival fibroblast following bacterial challenge is significantly higher, and stated that this manifest the capability of this cytokine in amplifying the local immune response and promoting inflammation in periodontium.

IL-8 could also be detected in the sites of healthy gingiva, since a small number of macrophages and mononuclear cells in the gingival tissue and neutrophils in the GCF can be found in clinically normal tissues. This could be related to the steady state of gingiva, considering that gingival sulcus is a site of permanent antigenic insult requiring the presence of neutrophils, macrophages and antigen presenting cells, which could be chemoattracted towards the gingival microenvironment by IL-8[9,30]Fredriksson et al 02 observed that IL-8 is found in higher levels in plasma, from patients in the quiescent phase of periodontal diseases. The pathogenic consequences of high plasma IL-8 levels in patients with periodontal diseases in its quiet phase can be attributed to the increased amount of this cytokine exuding into the periodontal lesion, attracting and priming neutrophils-indicating an active host immune response.

Such conflicting results may be related to the effect of endotoxins of bacteria such as Porphyromonas gingivalis on the cytokines. According to [29]Douglas et al 04, P.gingivalis creates an innate host defense defect that also disrupts neutrophil transit through the periodontium. The presence of P.gingivalis inhibits IL-8 expression thus disrupting the establishment of specific tissue gradient which results in misdirection of neutrophil migration (Chemotactic paralysis). This can contribute to the inverse relationship of IL-8 and PMN recruitment in the sites of periodontal break down. [1]Andrian et al 06 summarizes on the molecular cross talk between P.gingivalis and periodontal pathogens and suggests that such modulation of the mucosal epithelial barrier by the pathogenic bacteria is a critical step in the initiation and progression of periodontal disease.

Based on the above thoughts and discussions current study suggests the following, to more accurately assess the significance of IL-8 as a marker for future periodontal destruction.

- Clinical trials should be performed assessing IL-8 levels along with the microbiological markers, as this will give us evidence on the action of LPS from periodontopathogens on the expression and suppression of IL-8.
- Longitudinal studies should be performed on the concentration of IL-8 with relation to periodontal break down.
- Extensive research on the concentration of IL-8 should be done in relation to other factors that play a role in pathogenesis of periodontitis.

Clinical trials have indicated conflicting results on the level of IL-8 in periodontal health and disease. Increased expression of IL-8 observed from diseased sites in the current study confirms that this cytokine/chemokine is highly related to the inflammatory conditions of the periodontium. But this does not lay to rest the variance on IL-8, as a reliable marker that indicates the risk for future periodontal destruction. Extensive research should be done on a longitudinal basis assessing the level of this cytokine/chemokine for a better understanding on the aspect of using IL-8 as a reliable biomarker.

## CONCLUSION

IL-8 was assessed from GCF of healthy, plaque associated gingivitis and chronic periodontitis patients using ELISA assay. The increased expression of IL-8 observed from the diseased sites confirmed that this cytokine/chemokine is highly related to the inflammatory conditions of the periodontium. Studies on longitudinal basis to assess the level of this cytokine/chemokine should be done along with the evaluation of other modifying factors, for a better understanding on the aspect of using IL-8 as a reliable marker in the prediction of future periodontal destruction.

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